reveal a larger implant than measurements made on the 6th day in about ⅓ of the cases. Such growth does not occur when the hamsters are treated with Compound 48/80. The present evidence indicates that 48/80 has no effect either on mouse ascites tumor cells in the mouse or on hamster ascites tumor cells in the hamster cheek pouch. The evidence therefore does not support the explanation that the growth of tumor cells in the hamster cheek pouch is directly inhibited by 48/80. A more likely explanation appears to be that 48/80 enhances the ability of the hamster to reject a foreign tissue in its cheek pouch.

The increased growth of ascites tumor cells in the cheek pouch of hamsters treated with cortisone presumably results from an inhibition of antibody production by the hamster against the tumor cells(2). The fact that 48/80 does not affect inhibition of antibody production resulting from cortisone treatment suggests that 48/80 has a mode of action not directly associated with antibody production. Billingham *et al.*(3) has associated the persistence of homografts in the hamster cheek pouch with the occurrence in the cheek pouch of certain cells which prevent the arousal of the immunological defenses of the hamster.

The following interpretation, while speculative, seems consistent with the data. In the hamster cheek pouch a heterograft is surrounded by specific cells which render the heterograft less antigenic. These specific cells are sensitive to 48/80, so that treatment of the hamster with 48/80 results in prompt sensitization of the hamster, and rejection of the heterograft. Compound 48/80 is ineffective in cortisone-treated hamsters because, even though 48/80 may allow the heterograft to sensitize the hamster, the hamster's ability to respond to sensitization with antibody production is inhibited by cortisone.

Summary. Implants of mouse ascites tumor cells in cheek pouches of control hamsters showed progressive growth during the first 10 days in about ⅓ of the hamsters. In 48/80 treated hamsters, however, progressive growth of the implant did not occur. Compound 48/80 did not prolong the lives of mice inoculated intraperitoneally with mouse ascites tumor cells, nor of rats inoculated with Yoshida ascites tumor cells. Compound 48/80 did not inhibit the growth of hamster ascites tumor cells in the hamster cheek pouch, nor of mouse ascites tumor cells in the cheek pouches of cortisone treated hamsters. It is suggested that Compound 48/80 alters the cells of the hamster cheek pouch so that the hamster is more rapidly sensitized to foreign cells.

1. Hanks, J. H., Wallace, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 196.

2. Toolan, H. W., *Proc. N. Y. Acad. Sci.*, 1955, v59, 394.

3. Billingham, R. E., Ferrigan, L. W., Silvers, W. K., *Science*, 1960, v132, 1488.

Received April 18, 1962. P.S.E.B.M., 1962, v111.

Electrophoretic Determination of C-Reactive Protein Which Appears Following Surgery.* (27695)

M. HORNUNG AND T. M. MORRIS (Introduced by M. F. Shaffer)
Department of Microbiology, Tulane University School of Medicine, New Orleans, La.

The appearance of C-reactive protein (CRP) in serum represents a non-specific response to various types of physiological stress (1). Its absence from normal blood and its peculiar property of precipitating with the C (somatic) polysaccharide of pneumococcus raise the question of its immunological role. In this connection it would be useful to know

* The authors wish to express their appreciation for the cooperation of the surgical staff of Charity Hospital, New Orleans, La. and Dr. Oscar Creech, Dept. of Surgery, Tulane Medical School.

whether it is an alpha, beta, or gamma globulin.

Perlman(2) suggested that it is probably an alpha 1 globulin and there is general agreement that alpha globulins increase in conditions in which C-reactive protein occurs. In these conditions, however, Hedlund(3) and Jacox(4) found that alpha globulins continue to be elevated when CRP disappears. Wood (5) isolated crystalline CRP and found it moved as a beta globulin in free electrophoresis and as a gamma globulin in starch. Following zone electrophoresis of CRP-positive serum, Phillipson(6) found the serological activity associated with a fraction between the beta and gamma globulin peaks. Using an agar immunoelectrophoresis system, Bustamente(7) concluded that CRP was a gamma globulin. Reviewing the conflicting results obtained by immunodiffusion technics, Crowle (8) suggested that this procedure is probably unsatisfactory for determining the mobility of CRP. Shetlar(9) and Good(10) both found high levels of CRP produced by patients with agammaglobulinemia.

Usually CRP occurs in serum which is normal with respect to the total quantity of protein it contains. The amount of CRP produced is so small that its removal by absorption with C substance does not result in any significant change in the serum protein pattern. However, high CRP levels also occur within 24 hours following major surgery. In these instances the resultant blood loss may cause the normal blood proteins to be very much reduced in concentration. Therefore the newly appearing CRP constitutes a greater percentage of the total protein than is usually the case in CRP-positive serum. This paper reports the results obtained in a series of 10 surgical cases in which postoperative (CRP strongly positive) serum was compared with preoperative (CRP-negative) serum from the same individual. These were both compared with postoperative serum from which the CRP had been removed by absorption with C substance. In each case the 3 sera were simultaneously fractionated by paper electrophoresis, stained with bromphenol blue and examined, using an analytrol calibrated for the staining procedure employed. The serum fractions were calculated as percentages of total serum protein. A determination was made of the change in serum protein pattern which existed 24 hours after surgery, when the serum had converted from CRP-negative to CRP-positive (6 plus or more by Wood and McCarty's capillary tube method(11)). An attempt was also made to determine whether or not significant change in serum protein pattern occurred when CRP was removed from this postoperative serum.

Postoperative (CRP positive) sera were also fractionated with ammonium sulfate and the CRP containing fraction of each (concentrated 10 times) was examined electrophoretically for the presence of gamma globulin. The CRP was removed from these fractions and the resultant change in serum protein pattern determined. The CRP which had been removed was examined electrophoretically alone and in combination with CRP-negative preoperative serum of the same patient from whom it had been obtained.

At the same time, determinations were also made of the patient's temperature, total and differential leucocyte count, hematocrit and sedimentation rate in order to discover what correlation, if any, existed between high CRP levels and abnormalities in these findings.

Materials and methods. Test sera: Paired serum samples (preoperative and postoperative) were collected from patients undergoing major surgery (gastrectomy, thyroidectomy, hysterectomy) in Charity Hospital, New Orleans, La. The 10 cases selected for this study were those in which the preoperative serum was negative when tested with commercial CRP antiserum using Wood and McCarty's capillary tube method and which gave no precipitate with the C substance of pneumococcus. The postoperative serum in each case was strongly positive by both tests (6 plus or more with antiserum). These patients had not received blood during the operation nor in the interval following surgery before the postoperative specimen was drawn (approximately 20-24 hr).

Removal of CRP: 0.9 ml of the CRP-negative preoperative serum was placed in a first tube; 0.9 ml of the postoperative CRP-positive serum sample was placed in each of 2 ad-

TABLE I. Serum Protein Pattern Changes in a Typical Surgical Case. Effect produced by removal of CRP from CRP-positive postoperative specimen.

	% of total protein					
	Albumin	α 1	α 2	β	γ	Total
Preoperative (CRP-neg.)	61.8	1.9	6.0	9.0	21.3	100
Postoperative (CRP-pos. 9+)	50.0	5.2	13.3	9.5	22.0	100
C absorbed postoperative (CRP-neg.)	50.0	3.0	12.5	11.0	23.5	100

ditional tubes. To each of tubes 1 and 2 was added 0.1 ml of phosphate buffered (pH 7.4) saline which contained .01% $CaCl_2$; 0.1 ml of a 0.1% solution of C substance in this same buffered saline was added to Tube 3. (Preparation of the C substance will be described elsewhere.) After mixing, the 3 tubes were incubated at 37°C for 2 hrs, allowed to stand for 16 hrs at 4°C and centrifuged at 2000 rpm for 10 minutes. There was no precipitate in Tubes 1 or 2 but a precipitate of C-CRP complex was present in Tube 3. The supernate from each tube was removed and tested for CRP with CRP antiserum. Tubes 1 and 3 were negative. Tube 2 was strongly positive (6 plus or more).

Electrophoresis: 0.01 ml samples of each supernate were electrophoresed simultaneously on paper, using paper strips 30 cm long by 3 cm wide in Durrum cells containing barbital buffer at pH 8.6 and 0.1 ionic strength. A constant current of 2.5 ma (74 volts) was applied for 16 hrs at room temperature. The strips were then dried, stained with bromphenol blue and scanned in an analytrol calibrated for the staining procedure used (Spinco staining procedure B). The integrator units were counted and the value for each protein fraction was calculated as a percentage of the total. In some instances the bands representing beta, alpha 2 and alpha 1 fractions were eluted separately, each dissolved in 5 ml of Na_2CO_3 and read on a photelometer.

Preparation of serum fractions: 10 ml of CRP positive (6+) postoperative serum was diluted with an equal volume of saline. This solution was then made 50% saturated by addition of an equal volume of saturated ammonium sulfate at room temperature. The precipitate which formed was removed by centrifugation and designated fraction 1. The supernate was then made 75% saturated by addition of saturated ammonium sulfate at room temperature. The precipitate which formed was collected by centrifugation and designated fraction 2. Both fractions were redissolved separately, dialyzed against buffered saline in the cold until they gave negative barium chloride tests for sulfate. They were then both tested for the presence of CRP with CRP antiserum and with C substance. The volume of fraction 2 (which contained the CRP) was reduced to 1 ml by dialysis against polyvinylpyrrolidine in buffered saline in the cold. To one 0.4-ml sample of this solution was added 0.1 ml of a 0.05% solution of C substance. One-tenth ml of buffered saline was added to a second 0.4-ml sample. Both were incubated at 37° for 2 hrs, followed by 16 hrs at 4°-8°, at which time a precipitate of C-CRP complex formed in the C-treated sample. No precipitate formed in the saline treated sample. After centrifugation at 2000 rpm for 20 minutes both supernates were removed and 0.01 ml aliquots of each electrophoresed simultaneously as described above.

Preparation of CRP: The C-CRP precipitate of each specimen was redissolved in citrate and the CRP recovered according to the method of Wood(5). This CRP was dissolved in 0.1 ml of buffered saline, then electrophoresed alone and in combination with preoperative (CRP negative) serum of the same patient from whom it had been obtained. In the latter case, 0.02 ml of the CRP solution and 0.006 ml of serum were applied at the same point on the paper strip.

Results. All 10 paired serum samples showed the same changes in serum protein pattern. The results of a typical experiment are shown in Table I. In the preoperative serum taken immediately before the operation, alpha globulins (1 and 2) constitute 7.9% of total serum proteins. Twenty-four hours after

TABLE II. Serum Protein Values (%) Obtained for Thirty .01 ml Samples of the Same Normal Serum Electrophoresed Simultaneously.

	Albumin	α 1	α 2	β	γ	Total
Mean	65.5 ± 1.5	3.9 ±.3	7.7 ±.5	10.5 ±.3	12.4 ± 1.0	100
S. D.	1.1	.3	.3	.2	.8	100

operation the percentage of alpha globulins had risen to 18.5%. When the CRP was removed from this postoperative serum, the percentage of alpha globulins dropped to 15.5%. The major change occurred in the alpha 1 globulin fraction which rose from 1.9% preoperatively to 5.2% within 24 hrs following surgery and dropped to 3% when the CRP was removed. At the same time, the alpha 2 globulins rose from a preoperative level of 6% to a postoperative level of 13.3%. Following the absorption of CRP from this postoperative serum, the level of alpha 2 globulins dropped slightly to 12.5%, a drop of 0.8%, which was considered to be below the level of significance which was taken to be 1%. This figure (1%) was chosen after repeated experiments had shown that the method gave results which were reproducible at this level (Table II). The removal of CRP did not cause a reduction in any other serum fraction. The absorbed postoperative serum continued to show a high alpha globulin level (15.5% as opposed to 7.9% preoperatively), indicating the formation of other alpha globulins which were not C-reactive and which appeared to lie mostly in the alpha 2 region. Table III gives the results obtained in a typical experiment in which the beta, alpha 2 and alpha 1 bands were eluted separately in 5% Na_2CO_3 and read in a photelometer. In this case it can be seen that although the absolute values for beta and alpha 2 globulins were unchanged by absorption with C, there was a drop in alpha 1 amounting to 9.8% of the total of these 3 globulins.

TABLE III. Serum Protein Values for Beta, Alpha 2 and Alpha 1 Fractions Eluted in 5% Na_2CO_3.

	β		α 2		α 1		Total	
	P*	%	P	%	P	%	P	%
Non-absorbed	17	34	19	38	14	28	50	100
C-absorbed	17	38.6	19	43.2	8	18.2	44	100

* P = photelometer reading.

When the serum was fractionated, the fraction precipitated at 50% saturation with ammonium sulfate gave negative tests for CRP with both the antiserum and C substance. Fraction 2, which precipitated between 50% and 75% saturation with ammonium sulfate, was strongly positive by both tests. When this fraction was concentrated 10 times and a 0.01-ml sample electrophoresed, no gamma globulin could be detected (A of Fig. 1). Fig. 1B shows the electrophoretic pattern of a

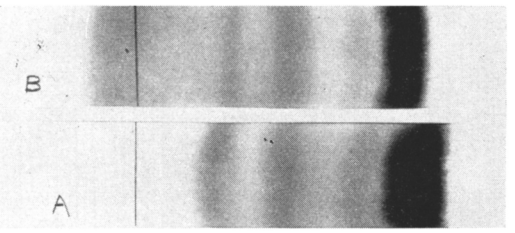

FIG. 1. Electrophoretic serum protein pattern of concentrated CRP containing fraction of CRP pos. (6+) serum. A, 0.01 ml of CRP containing fraction concentrated 10 times. B, 0.004 ml of preoperative CRP neg. serum of same patient unconcentrated. Cross bars in each case superimposed to mark origin.

0.004-ml sample of preoperative serum from the same individual, unconcentrated.

Absorption of CRP from fraction 2 resulted in a reduction of alpha globulins in terms of percent of total serum protein (Table III). Table IV shows the absolute and relative globulin values of fraction 2 before and after removal of CRP. Here it can be seen that although the absolute value of beta globulin remained the same following removal of CRP, there was a reduction in the absolute value of alpha globulins which amounted to 15.4% of the total globulin present.

When CRP was electrophoresed alone a small percentage of it (as measured by bromphenol blue staining) moved 6.5 mm, the same distance moved by alpha globulins in the neighboring strips. However, the major portion of the CRP, whether alone or in combination with serum, precipitated at the ori-

TABLE IV. Serum Protein Values (%) Obtained for Non-absorbed and C-absorbed CRP-Pos. Fraction.

	Albumin	α 1	α 2	β	γ	Total
Non-absorbed	74.8	12.1	4.8	8.3	0	100
C-absorbed	76.2	10.2	4.3	9.3	0	100

gin. The CRP appeared to have been somewhat denatured in the isolation process which involved treatment for 4 days with 75% saturated ammonium sulfate at 37° followed by prolonged dialysis.

With respect to other determinations, the temperature was normal at both times blood was drawn and total leucocyte counts were within normal range. Differential leucocyte counts showed a marked increase in immature cells of all types. The percentages of polymorphonuclear types, lymphocytes and monocytes were within normal limits but there was a reduction in eosinophils (0.125% to 0.25% as compared with a normal of 2% to 5%). Hematocrit values were reduced in the postoperative specimens and the corrected sedimentation rates ranged from 9 mm to 20 mm (Wintrobe tube).

Discussion. The failure to detect gamma globulin in the CRP containing fraction of postoperative serum even when this fraction is concentrated 10 times would seem to be conclusive evidence that the CRP which appears following surgery is not a gamma globulin. On the other hand, removal of CRP from postoperative serum or from the concentrated CRP containing fraction of this serum results in a consistent drop in the alpha globulins of this serum or fraction and seems to indicate that this CRP is an alpha globulin.

Since CRP is regularly isolated from serum or other body fluids in the fraction which precipitates at 50% to 75% salt saturation, it would seem logical to find that it is an alpha globulin. However, as noted above, when CRP-positive serum has been fractionated by electrophoretic or other methods and the fractions tested serologically, the CRP activity has been found in various other areas—in the beta fraction, between the beta and gamma fractions or in the gamma fraction. Two possible explanations suggest themselves. During other experimental work in this laboratory, it has been frequently observed that, although CRP retains its antigenic specificity over long periods of time, it is physically quite unstable. It is well known that it can be precipitated by dialyzing positive serum against water containing small traces of calcium. It also adsorbs readily to filters of various types to such extent that attempts to clarify cloudy positive serum by filtration through porcelain or sintered glass invariably result in loss of CRP activity in the filtered serum. This tendency to precipitate (as evidenced also in the present work) and to be adsorbed on to various substances might account for finding the active material at or close to the origin following electrophoretic separation in supportive media of different types. Another explanation might be that CRP represents a conjugated protein consisting of a carrier protein in loose combination with some other substance which is separated from it by electrolysis and is subsequently found in other, slower moving fractions. It has recently been possible to prepare rather large amounts of CRP in this laboratory and experiments designed to explore the latter hypothesis are in progress.

TABLE V. Globulin Values Obtained for Non-absorbed and C-absorbed CRP-Pos. Fraction.

	α 1 & 2		β		γ		Total	
	*TC	%	TC	%	TC	%	TC	%
Non-absorbed	91	67	45	33	0	0	136	100
C-absorbed	70	61	45	39	0	0	115	100

* Tooth count by analytrol.

The presence of high CRP levels in conjunction with normal temperature, sedimentation rate and total white count show that, following surgery, CRP appears before these other indices of tissue reaction become abnormal. The immaturity of the leucocytes and eosinopenia were expected findings following this particular type of physiological stress. Although they occurred in 100% of

50 CRP-positive postoperative sera examined, it would be necessary to study the blood picture in other CRP-positive conditions before their relevance could be established.

Summary. A study was made of the sera of surgical patients in which preoperative serum was negative for CRP and postoperative serum was strongly CRP-positive. In all cases there was a marked elevation in the alpha globulin fraction of the positive postoperative serum. When the CRP was removed from the postoperative serum, there was a significant reduction in alpha globulin, chiefly in the alpha 1 globulin fraction. When this CRP-positive serum was fractionated with ammonium sulfate, the fraction containing CRP contained no gamma globulin. It was concluded that CRP which appears following surgery is an alpha globulin.

1. Roantree, R. J., Rantz, L. A., *A.M.A. Arch. Int. Med.*, 1955, v96, 674.
2. Perlman, E., *J. Exp. Med.*, 1942, v77, 97.
3. Hedlund, P., *Acta Med. Scand.*, 1948, v131, 147.
4. Jacox, R., *N. Y. State J. Med.*, 1956, v56, 672.
5. Wood, H., McCarty, M., *J. Exp. Med.*, 1954, v100, 71.
6. Phillipson, L., *Acta Pediat.* (Uppsala), 1957, v46, 70.
7. Bustamente, V., *Revista Clinica Espanola,* 1957, v67, 9.
8. Crowle, A. J., *Immunodiffusion,* Academic Press, 1961, p116.
9. Shetlar, M. R., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 107.
10. Good, R., *Bull. U. Minn. Hosp.*, 1954, v26, 1.
11. Wood, H., McCarty, M., *J. Clin. Invest.*, 1951, v30, 616.

Received April 18, 1962. P.S.E.B.M., 1962, v111.

Effect of Gamma Irradiation on Pigs Fed Low Vitamin A Rations.* (27696)

T. N. MEACHAM, T. J. CUNHA, G. E. COMBS, H. D. WALLACE, A. C. WARNICK, R. L. SHIRLEY AND C. F. SIMPSON†
Department of Animal Science, University of Florida Agricultural Experiment Station, Gainesville

Little information is available concerning the relationship of irradiation of large animals and nutritional stress. It is not known whether a nutritional deficiency in such animals might have an effect on the severity of radiation damage. A detailed chronological description of the symptoms associated with a single, acute low level irradiation of swine is also lacking. The present study was conducted to determine the effect of low level irradiation on Vit. A low and control pigs and to observe the development of lesions due to radiation damage.

Methods and materials. In a preliminary study to establish dosage levels, 10 pigs were irradiated at 200-1,000 r as shown in Table I. These crossbred pigs were 8 weeks old and weighed approximately 30 lb.

In the present study, 60 crossbred male and female pigs, weaned at 2 weeks of age, were allotted to 2 groups of 30 on the basis of weight, sex and litter. The pigs were placed in concrete fattening pens with feed and water supplied *ad libitum*. One group of pigs received a basal ration (Table II) containing 0.341 mg of carotene (equivalent to 170.5 I.U. of Vit. A) per pound of total feed. The other group received the same basal ration supplemented with 2,000 I.U. of Vit. A acetate per pound of feed.

The pigs were weighed weekly and blood samples were taken from the anterior vena cava at 2-3-week intervals for Vit. A analysis. Two pigs were removed from the Vit. A low group at 11 weeks due to abdominal hernia.

At the end of 13 weeks, each of the 2 groups was divided into 3 lots. Two of the lots were composed of 10 pigs which most closely approached the average body weight of their respective ration group. The remain-

* Florida Agri. Exp. Station Journal Series No. 1448.

† C. F. Simpson, Dept. of Vet. Sci.